The elusive link between vitamin D levels, supplementation and ill health: time to up the game?

Prof Declan Naughton
Talk Content

• Progress in vitamin D research
• Case to improve measurement capability
• Questions to be addressed by advanced assays
• Extending field to more forms – help to understand the vitamin D metabolome
Deeb et al. Nature Reviews Cancer 7, 684-700
Vitamin D clinical research areas

- Low endogenous levels lead to impaired health, reproduction and growth, and contribute to disease onset and/or progression.
Vitamin D clinical research areas

- Low endogenous levels lead to impaired health, reproduction and growth, and contribute to disease onset and/or progression

- Supplementing with vitamin D will counter these effects
Scopus – “Vitamin D”
Subject area  (101,721 documents)

Document type
## Vitamin D in health and disease: Current perspectives

Ran Zhang\(^1\) and Declan P Naughton\(^2\)*

### Table 3 Systematic reviews on Vitamin D for prevention or treatment (in chronological order)

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of studies</th>
<th>Type</th>
<th>Doses</th>
<th>Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular events</td>
<td>17</td>
<td>Prospective</td>
<td>~1000IU</td>
<td>S not SS</td>
<td>162</td>
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<tr>
<td>Cardiometabolic outcomes</td>
<td>31</td>
<td>Observ/Trial</td>
<td>Range</td>
<td>No effect</td>
<td>163</td>
</tr>
<tr>
<td>Fracture prevention</td>
<td>7</td>
<td>Randomised</td>
<td>10-20 mcg + Ca</td>
<td>No effect Reduced risk</td>
<td>164</td>
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<tr>
<td>Physical performance</td>
<td>8</td>
<td>Observational</td>
<td>-</td>
<td>Positive (5/8)</td>
<td>165</td>
</tr>
<tr>
<td>Kidney disease (-dialysis)</td>
<td>16</td>
<td>RCT</td>
<td>-</td>
<td>Uncertain</td>
<td>166</td>
</tr>
<tr>
<td>Kidney disease (+dialysis)</td>
<td>60</td>
<td>RCT</td>
<td>-</td>
<td>Uncertain</td>
<td>167</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>3</td>
<td>(q)RCT</td>
<td>800-1600IU</td>
<td>Reduced risk</td>
<td>168</td>
</tr>
<tr>
<td>Risk of falling</td>
<td>8</td>
<td>RCT</td>
<td>700-1000IU 200-600IU</td>
<td>Reduced risk</td>
<td>169</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11</td>
<td>RCT</td>
<td>-</td>
<td>S - SS</td>
<td>170</td>
</tr>
<tr>
<td>Risk of falling</td>
<td>111*</td>
<td>RT or interv.</td>
<td>-</td>
<td>No effect</td>
<td>171</td>
</tr>
<tr>
<td>Fracture prevention</td>
<td>45</td>
<td>(q)RCT +Ca</td>
<td>-</td>
<td>No effect</td>
<td>172</td>
</tr>
<tr>
<td>Risk of Type 1 diabetes</td>
<td>5</td>
<td>Observational</td>
<td>-</td>
<td>S not SS</td>
<td>172</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>52</td>
<td>Intervention</td>
<td>-</td>
<td>Decrease</td>
<td>173</td>
</tr>
<tr>
<td>Mortality</td>
<td>18</td>
<td>RCT</td>
<td>2300-2000IU</td>
<td>Uncertain</td>
<td>174</td>
</tr>
<tr>
<td>Risk of fall/fracture</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>Decrease (trend)</td>
<td>175</td>
</tr>
<tr>
<td>Risk of fracture</td>
<td>12</td>
<td>RCT</td>
<td>700-800IU 400IU</td>
<td>Decrease (trend)</td>
<td>176</td>
</tr>
<tr>
<td>Risk of fall</td>
<td>10</td>
<td>RCT</td>
<td>-</td>
<td>Decrease</td>
<td>177</td>
</tr>
<tr>
<td>Bone density Fracture</td>
<td>17</td>
<td>RCT</td>
<td>-</td>
<td>Uncertain Reduction</td>
<td>178</td>
</tr>
</tbody>
</table>

S not SS: Small effect - not statistically significant (sub-groups are discounted); RCT: Randomised controlled trial [(q) - quasi]; * Multiple factor study.

40 IU = 1 mcg
Lack of progress

• What are the issues?
Need for **application** of accurate assays
Complex assay development

7-Dehydrocholesterol \( \xrightarrow{\text{SKIN}} \text{Vitamin D3} \xrightarrow{\text{LIVER}} \text{25OHD3} \xrightarrow{\text{KIDNEY}} \text{1α,25(OH)\textsubscript{2}D3} \)

\text{(390-315 nm) UVB} 25-hydroxylase 1α-hydroxylase
Complex assay development

7-Dehydrocholesterol $\xrightarrow{SKIN}^{(390-315\text{ nm})\text{UVB}}$ Vitamin D3 $\xrightarrow{LIVER}$ 25-hydroxylase $\xrightarrow{\text{LIVER}}$ 25OHD3 $\xrightarrow{\text{KIDNEY}}$ 1α-hydroxylase $\xrightarrow{\text{KIDNEY}}$ 24,25(OH)2D3 $\xrightarrow{\text{KIDNEY}}$ 1α,25(OH)2D3

Vitamin D2

Vitamin D3
Epimer forms for D3 (& D2)
1 ng/mL ≈ 2.5 nmol
Need to have a consensus on required levels

Problems with defining vitamin D deficiencies

Zittermann (2003) - “optimum vitamin D status” between 100-250 nmol.L-1

US Institute of Medicine (IoM) define inadequate vitamin D status as <50 nmol.L-1 suggesting potential adverse events when levels are >125 nmol.L-1.

The Scientific Advisory Committee on Nutrition (SACN) and the Food Standards Agency (FSA) of the UK define vitamin D deficiency as <25 nmol.L-1.
What level is required – for each condition?

<table>
<thead>
<tr>
<th>STUDY GROUP</th>
<th>25OHD3 / 1,25(OH)_2D3</th>
<th>25OHD2 / 1,25(OH)_2D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td>230</td>
<td>-</td>
</tr>
<tr>
<td>Healthy</td>
<td>1024</td>
<td>105</td>
</tr>
<tr>
<td>Type 1 Diabetes</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>1257</td>
<td>62</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>406</td>
<td>349</td>
</tr>
</tbody>
</table>

Careful bioregulation of active forms?
Is the measurement approach acceptable?

Exploring the Role of Vitamin D in Type 1 Diabetes, Rheumatoid Arthritis and Alzheimer's Disease: New Insights from Accurate Analysis of Ten Forms
Iltaf Shah, Andrea Petroczi and Declan P Naughton

Large range of variables – season, diet, sun exposure, etc.

Studies based on blood tests – often single measurements – are fallible.

Is there an alternative?
New hair test to determine vitamin D status?

Hair growth = ca. 1 cm per month

No risk of infection

Facile transport to specialised laboratory

One test covers 3 – 5 months

P. Kintz (2007)
New hair test to determine vitamin D status?

### Young adults (n = 77, 36.4% male)

<table>
<thead>
<tr>
<th></th>
<th>December - March</th>
<th>June - September</th>
<th>Seasonal variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D level</td>
<td>0.4645 ± 0.048 pg/mg ± SE</td>
<td>0.9474 ± 0.141 pg/mg ± SE</td>
<td>[F(1,75) = 9.877, p = .002]</td>
</tr>
<tr>
<td>No gender difference</td>
<td>[F(1,75) = 0.290, p = .592]</td>
<td>No interaction effect</td>
<td>[F(1,75) = 0.040, p = .843]</td>
</tr>
</tbody>
</table>

P. Kintz (2007)
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• Progress in vitamin D research
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Advanced assays help improve understanding of the vitamin D metabolome

1. Biological activity of analogues?
Improving understanding of the vitamin D metabolome

1. Biological activity of analogues?
2. Potential regulatory roles of analogues -
Improving understanding of the vitamin D metabolome

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2. Potential regulatory roles of analogues -
   
   **Enzyme inhibition during activation or catabolism with multiple forms present?**
Improving understanding of the vitamin D metabolome

1. Biological activity of analogues?

2. Potential regulatory roles of analogues -
   - Enzyme inhibition during activation or catabolism with multiple forms present?
   - Affect binding to VDBP?
Improving understanding of the vitamin D metabolome

1. Biological activity of analogues?

2. Potential regulatory roles of analogues -
   - Enzyme inhibition during activation or catabolism with multiple forms present?
   - Affect binding to VDBP?
   - Affect binding to Receptor?
Scopus -
“Vitamin D” AND Epimer

880 results
Role for epimers?

1α,25(OH)2-3-Epi-Vitamin D3, a Natural Physiological Metabolite of Vitamin D3: Its Synthesis, Biological Activity and Crystal Structure with Its Receptor

Ferdinand Molnár1,2, Rita Sigüeiro3, Yoshiteru Sato1, Clarisse Araujo3, Inge Schuster4, Pierre Antony1, Jean Peluso5, Christian Muller5, Antonio Mouriño3, Dino Moras1, Natasha Rochel1*

1 Institut de Genetique et de Biologie Moleculaire et Cellulaire (IGBMC), Institut National de Santé et de Recherche Medicale (INSERM) U964/Centre National de Recherche Scientifique (CNRS) UMR 7104/Université de Strasbourg, Illkirch, France, 2 School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland, 3 Departamento de Quimica Organica, Universidad de Santiago de Compostela and Unidad Asociada al CSIC, Santiago de Compostela, Spain, 4 Institute of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria, 5 Faculty of Pharmacy, Institut Gilbert Laueart, UMR 7175 CNRS, University of Strasbourg, Illkirch, France
Figure 1. Mean concentration of (A) maternal and (B) neonatal vitamin D forms.
1: 25OHD2, 2: 25OHD3, 3: 3-epi-25OHD2, 4: 3-epi-25OHD3, 5: 1α,25(OH)₂D₃
Frequency distribution of maternal (A) and neonate 25(OH)D levels*

*excludes epimers – typically 25%
Neonate study

- 85% of mothers had deficient or insufficient levels of vitamin D

- Hierarchical linear regression models revealed:
  - maternal characteristics explained 12.2% of the neonatal 25(OH)D,
Neonate study

• 85% of mothers had deficient or insufficient levels of vitamin D

• Hierarchical linear regression models revealed:
  • maternal characteristics explained 12.2% of the neonatal 25(OH)D,
  • maternal 25(OH)D concentrations explained 32.1%,
85% of mothers had deficient or insufficient levels of vitamin D.

Hierarchical linear regression models revealed:
- maternal characteristics explained 12.2% of the neonatal 25(OH)D,
- maternal 25(OH)D concentrations explained 32.1%,
- while epimers contributed an additional 11.9%.
Exploring the Role of Vitamin D in Type 1 Diabetes, Rheumatoid Arthritis and Alzheimer's Disease: New Insights from Accurate Analysis of Ten Forms
Iltaf Shah, Andrea Petroczi and Declan P Naughton

The key findings are:

- **the 23R,25(OH)₂D₃ form was quantified for the first time**
  (healthy = 0.427±0.633 nmol/L; combined disease = 0.395±0.483 nmol/L),

- **the 3-epi-25OHD₃ metabolite was found in all groups with significantly higher concentration in the diseased samples**
  (healthy = 6.093±6.711 nmol/L; combined disease 22.433±13.535 nmol/L, t(52.5)=−6.411; p<0.001),

J Clin Endocrinol Metab  2014 Jan 13:jc20132872
• Discriminant function analysis using concentrations of 23R,25(OH)$_2$D$_3$, 25OHD$_2$ and 3-epi-25OHD classified 94.4% (91.7% in cross-validation) of the cases correctly.
• ROC analysis showed good sensitivity and specificity for using 3-epi-25OHD concentration to predict disease status (AUC = 0.880, p < 0.001).

Role for other analogues?

- Interacts with VDR
- Translocation to nucleus
- Stimulate VDRE-reporter activity
- Regulate VDR downstream genes
- Inhibits production of inflammatory markers
Improving understanding of the vitamin D metabolome

1. Biological activity of analogues?

2. Potential regulatory roles of analogues -
   - Enzyme inhibition during activation or catabolism with multiple forms present?
   - Affect binding to VDBP?
   - Affect binding to Receptor?
Summary

• Advanced assays have helped to
  – Provide rigour to Vitamin D research
  – Open up new questions about the wider range of forms

• A wider range of forms are now measured in a growing number of studies

• Other forms appear to have biological activity
Conclusions

• Advanced assays should be applied

• Other forms warrant further study as potential bio-regulators of vitamin D

• Suggestion to try supplementing varied forms
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Ran Zhang
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